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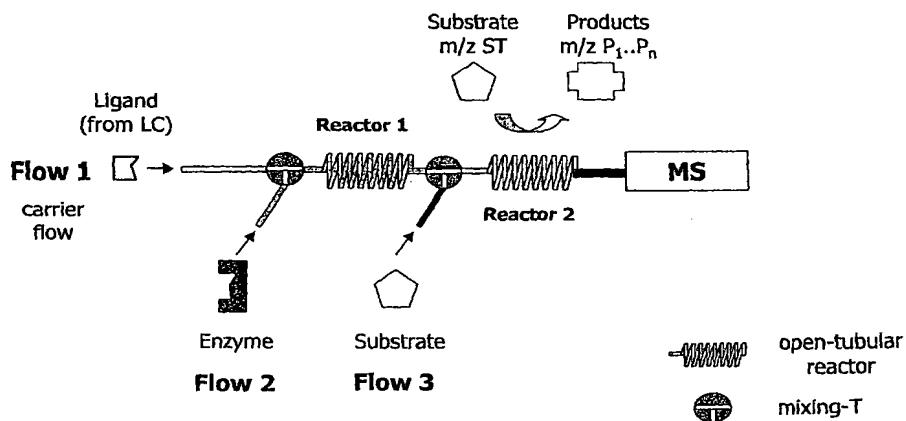
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CONTINUOUS-FLOW ENZYME ASSAY WITH MASS SPECTROMETRY DETECTION

**On-line enzyme MS assay – System 1**



(57) Abstract: The present invention relates to an on-line detection method comprising the steps of: contacting an effluent of a fractionation step with a controlled amount of an enzyme; allowing the enzyme to interact with analytes suspected to be present in the effluent; addition of a controlled amount of a substrate for said enzyme; allowing a reaction of the enzyme with the substrate providing one or more modified substrate products; and detection of unreacted substrate, or a modified substrate product using a mass spectrometer.

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## INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12Q1/00 C12Q1/48 G01N33/68 G01N35/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
 IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, MEDLINE, PAJ, WPI Data, COMPENDEX, INSPEC, FSTA

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>INGKANINAN K ET AL: "High-performance liquid chromatography with on-line coupled UV, mass spectrometric and biochemical detection for identification of acetylcholinesterase inhibitors from natural products"  <i>JOURNAL OF CHROMATOGRAPHY, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 872, no. 1-2, March 2000 (2000-03), pages 61-73, XP004190228 ISSN: 0021-9673 cited in the application the whole document page 67, right-hand column, paragraph 2 abstract page 64; figure 1C</i></p> <p>---</p> <p>-/-</p>	1-12

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 November 2003

Date of mailing of the international search report

04/12/2003

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## INTERNATIONAL SEARCH REPORT

PCT/NL 03/00408

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>LANGRIDGE JAMES I ET AL: "Analysis of cyclic nucleotide-related enzymes by continuous-flow fast-atom bombardment mass spectrometry." RAPID COMMUNICATIONS IN MASS SPECTROMETRY, vol. 7, no. 4, 1993, pages 293-303, XP008010052 ISSN: 0951-4198 cited in the application abstract page 293, right-hand column, line 5 -page 294, left-hand column, line 6 page 300, right-hand column, paragraph 3 -page 302, left-hand column, paragraph 1 ---</p>	1-11
Y	<p>WO 02 37111 A (IRTH HUBERTUS ; VRIJE UNIVERSITEIT VAN AMSTERDAM (NL)) 10 May 2002 (2002-05-10) the whole document page 3, line 13 - line 15 page 10, line 28 -page 11, line 3 ---</p>	1-12
Y	<p>ZHANG BOYAN ET AL: "Frontal affinity chromatography coupled to mass spectrometry for screening mixtures of enzyme inhibitors." ANALYTICAL BIOCHEMISTRY, vol. 299, no. 2, 15 December 2001 (2001-12-15), pages 173-182, XP002219168 ISSN: 0003-2697 cited in the application the whole document page 178; figure 3F page 179, left-hand column, line 8 - line 20 abstract page 174, right-hand column, paragraph 3 -page 175, right-hand column, paragraph 1 ---</p>	1-12
X	<p>CRAIG A GREY ET AL: "Monitoring protein kinase and phosphatase reactions with matrix-assisted laser desorption/ionization mass spectrometry and capillary zone electrophoresis: Comparison of the detection efficiency of peptide-phosphopeptide mixtures." BIOLOGICAL MASS SPECTROMETRY, vol. 23, no. 8, 1994, pages 519-528, XP008010048 ISSN: 1052-9306 abstract ---- -/-</p>	13

## INTERNATIONAL SEARCH REPORT

PCT/NL 03/00408

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MURRAY K J ET AL: "USE OF A SYNTHETIC DODECAPEPTIDE (MALANTIDE) TO MEASURE THE CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY RATIO IN A VARIETY OF TISSUES" BIOCHEMICAL JOURNAL, PORTLAND PRESS, LONDON, GB, vol. 267, 1 May 1990 (1990-05-01), pages 703-708, XP000945069 ISSN: 0264-6021 the whole document	13
X	KHOURI H E ET AL: "A MODEL TO EXPLAIN THE PH-DEPENDENT SPECIFICITY OF CATHEPSIN B-CATALYZED HYDROLYSES" BIOCHEMICAL JOURNAL, vol. 275, no. 3, 1991, pages 751-758, XP008025017 ISSN: 0264-6021 the whole document	13

**INTERNATIONAL SEARCH REPORT****PCT/NL 03/00408****Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
  
2.  Claims Nos.: 13 (in part) because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
see FURTHER INFORMATION sheet PCT/ISA/210
  
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

**1. Claims: 1-11 (fully), 13 (partially)**

Mass spectrometry coupled on-line detection method of enzyme activity in an effluent of a fractionation step by detection of unreacted substrate or modified substrate product.

**2. Claims: 12 (fully), 13 (partially)**

Mass spectrometry on-line detection method of different fractionation effluents to which enzyme and substrate are added using a multiple-inlet unit.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 13 (in part)

Present claim 13 relate to a compound defined by reference to a desirable characteristic or property, namely being detectable by a the method of any of claims 1-12.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds phosphorylated malantide, phosphorylated kemptide and the hydrolysis products of the compound Z-FR-AMC, mentioned in examples 1,2 and 3, respectively.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

## INTERNATIONAL SEARCH REPORT

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Patent document cited in search report	Publication date		Patent family member(s)	Publication date
WO 0237111	A	10-05-2002	EP 1202056 A1	02-05-2002
			AU 2420102 A	15-05-2002
			EP 1330648 A2	30-07-2003
			WO 0237111 A2	10-05-2002